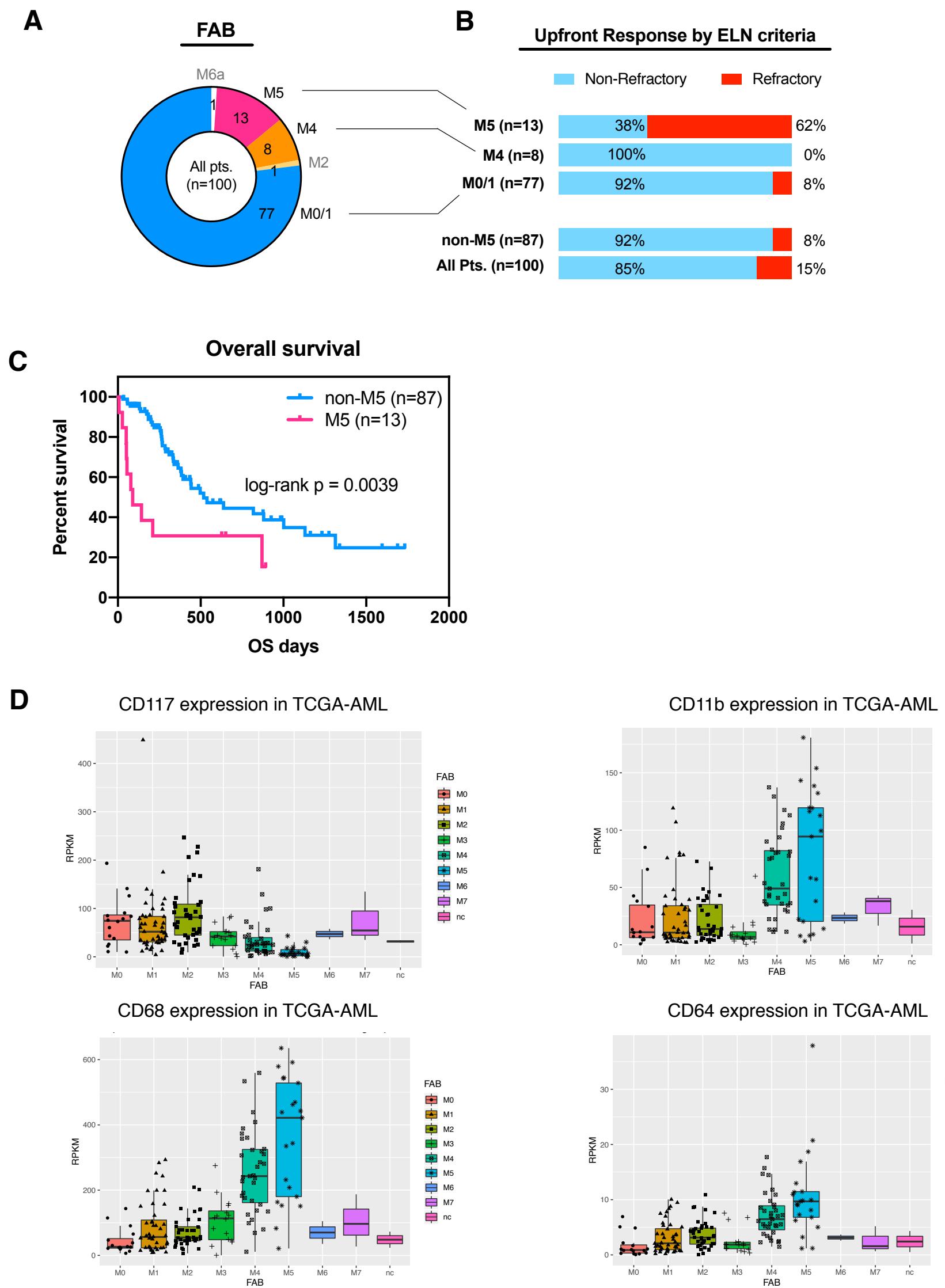


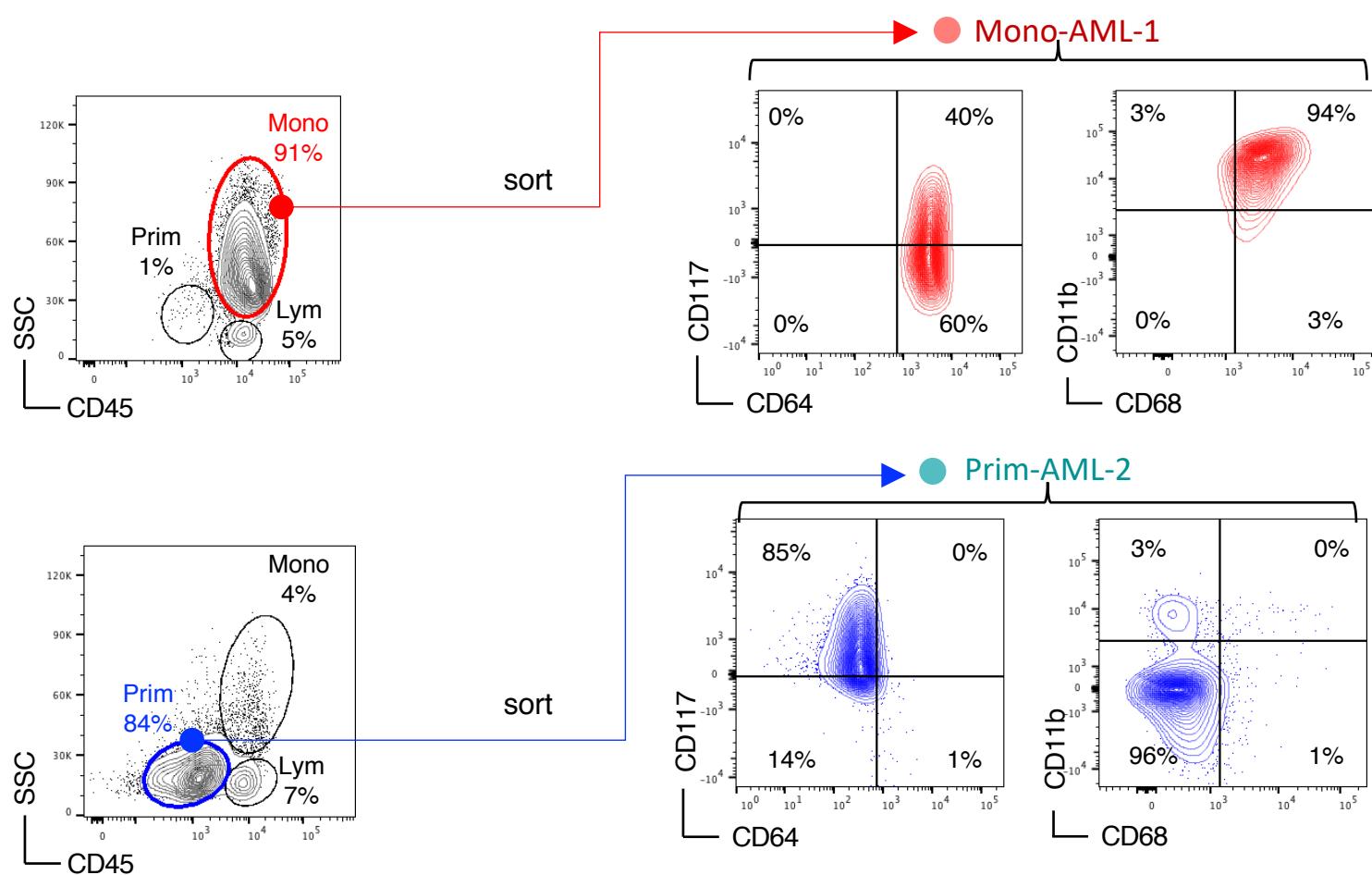
Supplementary Figure S1



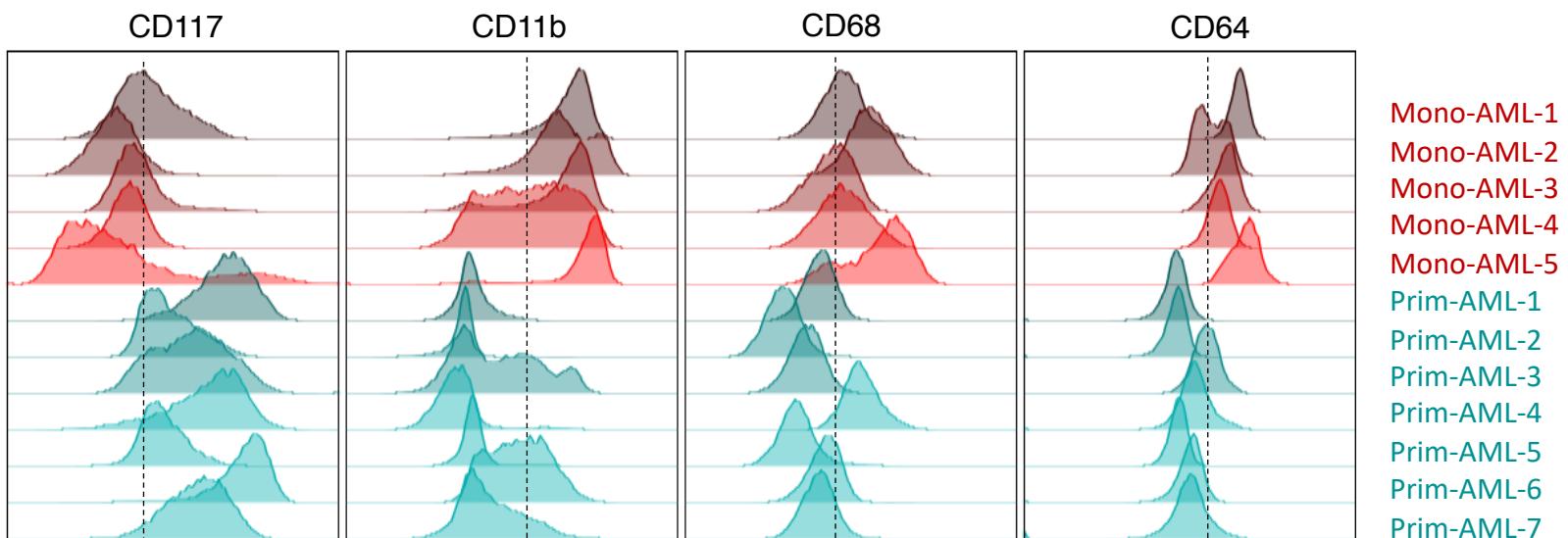
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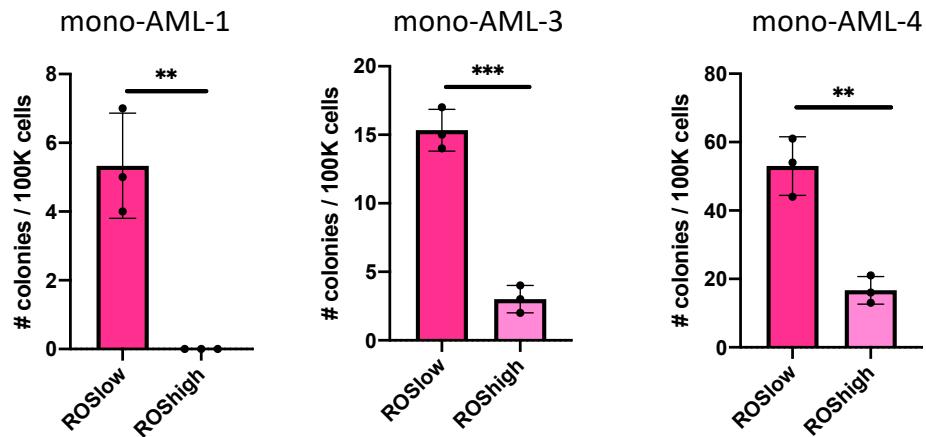
E



F



G



Supplementary Figure S1. AMLs with monocytic differentiation have poor clinic response and intrinsic resistance to venetoclax + azacitidine.

A, A circular pie chart showing number of patients identified in different FAB subclasses. Total number of patients is 100.

B, Bar graphs showing percentage of patients had refractory or non-refractory responses to VEN+AZA therapy according to the ELN criteria.

C, A Kaplan-Meier survival plot showing overall survival in M5 vs non-M5 patients. Log-rank p value is reported.

D, Box plots showing mRNA expression of primitive marker gene CD117, monocytic marker gene CD11b, CD68 and CD64 in different FAB subtypes in the TCGA-AML dataset. Each dot represents a unique AML patient specimen. Box represents median +/- interquartile.

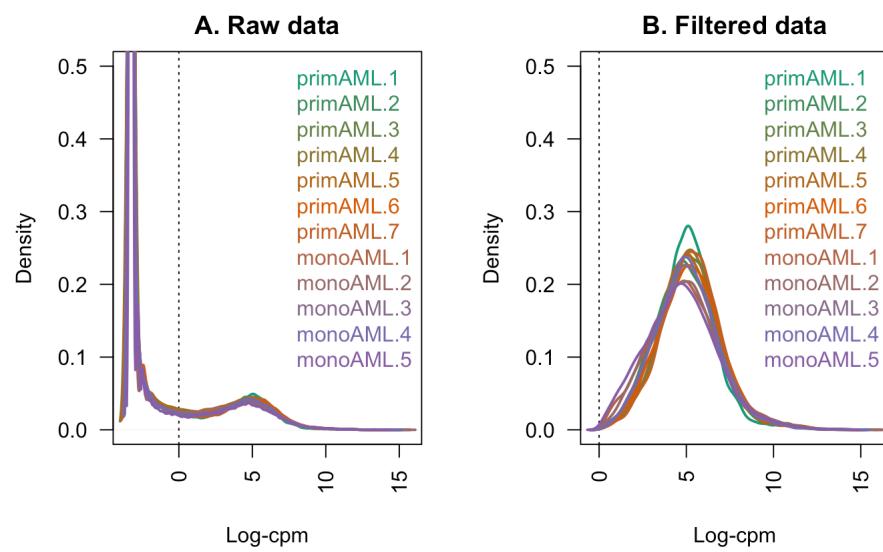
E, Representative flow plots showing gating strategies for sorting mono and prim AMLs. The mono-AMLS are CD45-bright/SSC-high/CD117-/CD64+/CD11b+/CD68+ (red), the prim-AMLS are CD45-medium/SSC-low/CD117+/CD64-/CD11b-/CD68- (blue).

F, Histograms showing relative intensity of each antigen in mono-AML (N=5) and prim-AML (N=7).

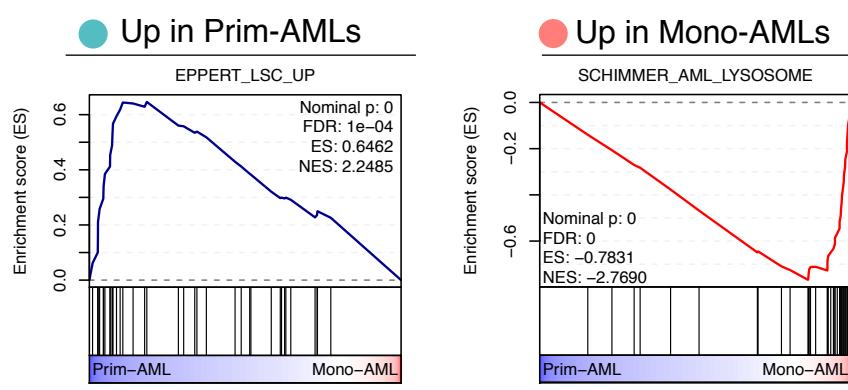
G, Results of Colony Forming Unit (CFU) assay comparing stem/progenitor function of ROS-low vs ROS-high subpopulations of mono-AML. Mean +/- SD. Two-tailed, unpaired t-test.

Supplementary Figure S2

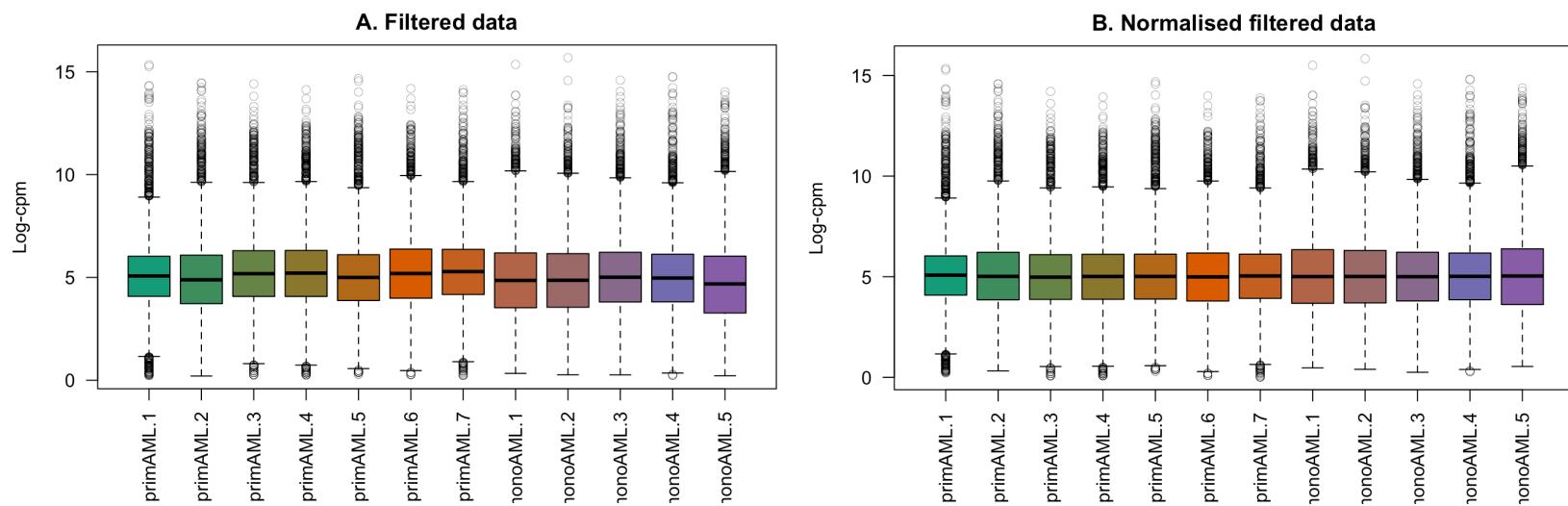
A



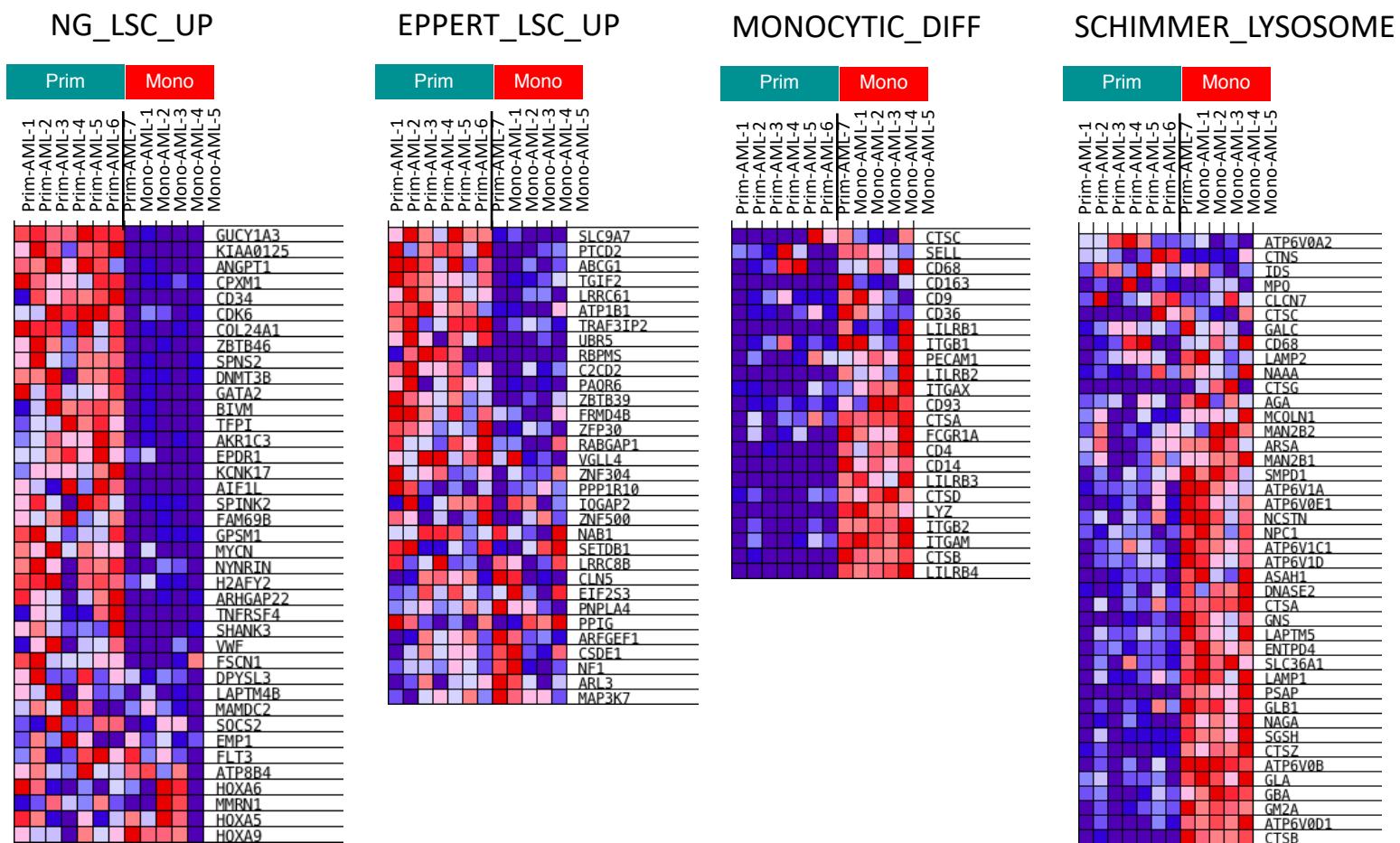
C



B



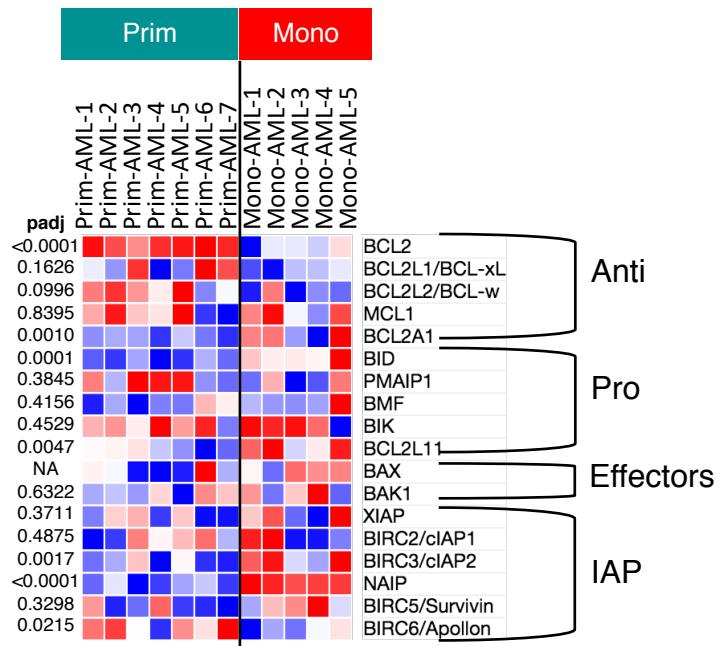
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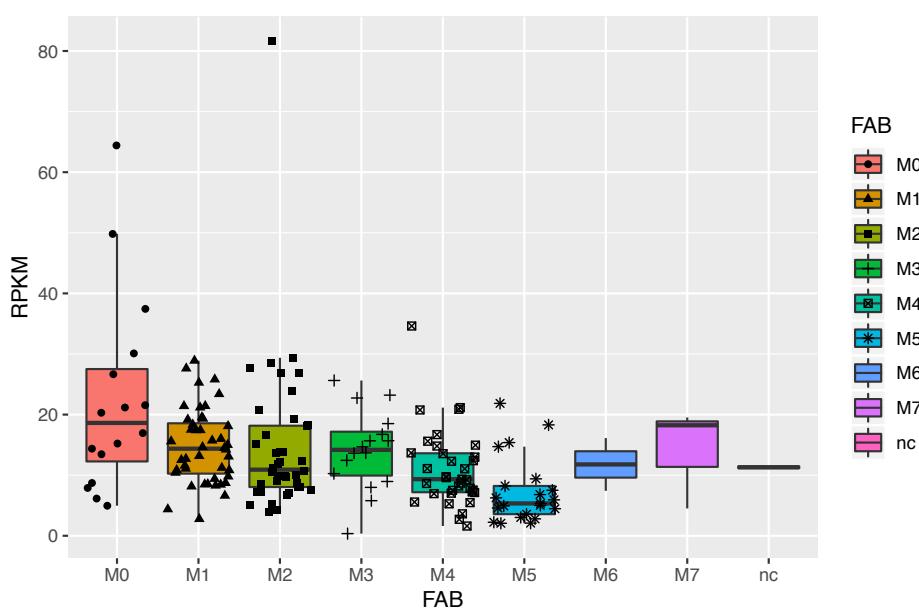
Supplementary Figure S2

continued

E Prim Mono

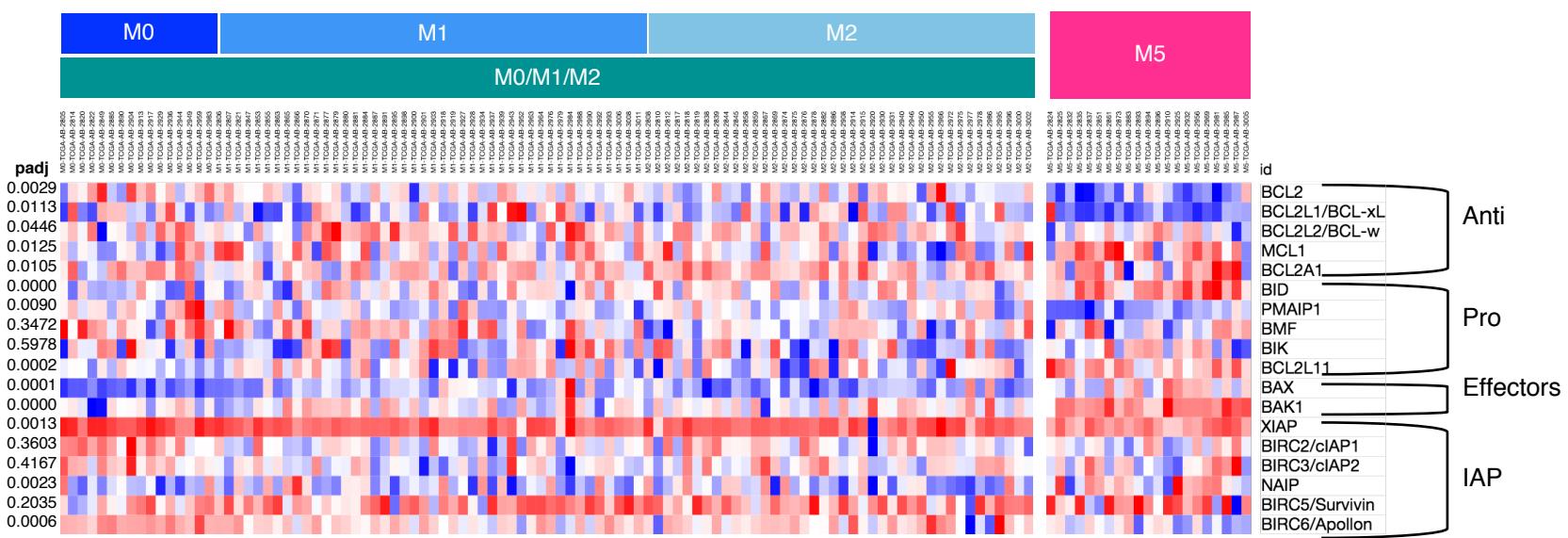


F BCL2 expression in TCGA-AML



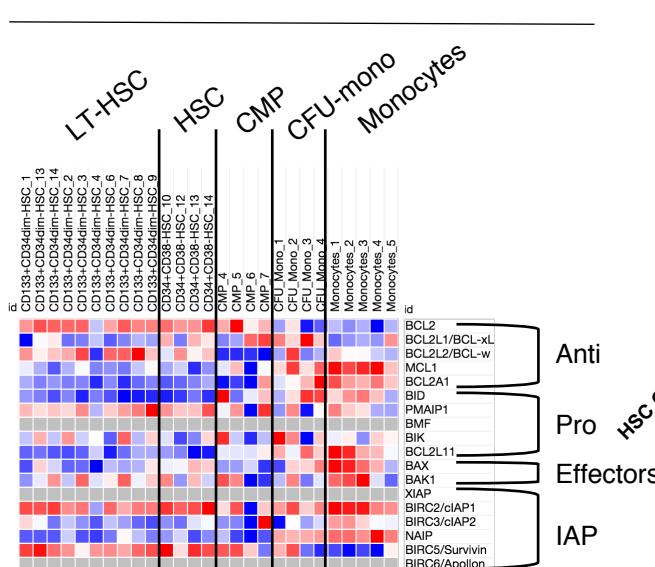
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TCGA-AML

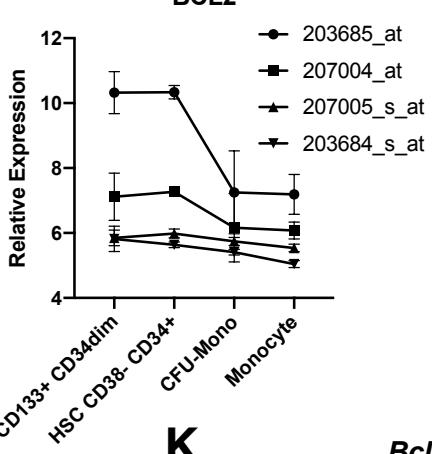


H

Human normal hematopoiesis

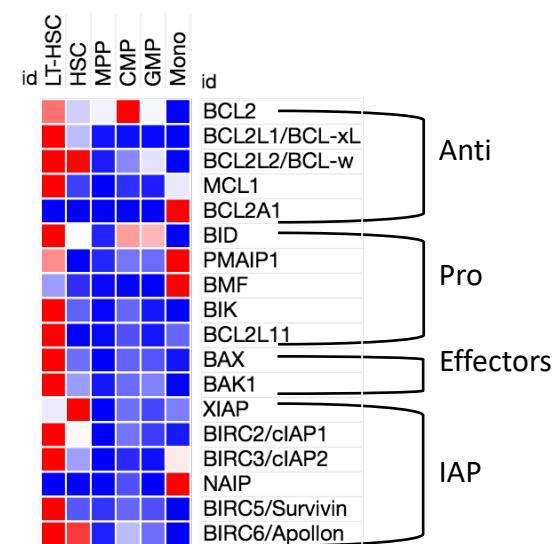


I BCL2

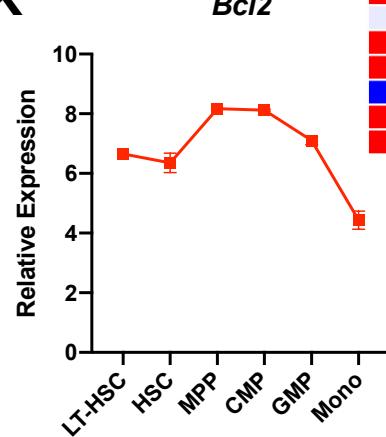


J

Murine normal hematopoiesis



K



Supplementary Figure S2. Loss of BCL2 is a conserved mechanism during both normal and malignant monocytic differentiation in both humans and mice.

A, Distribution of log-cpm (counts per million) of all genes before and after low count filtration.

B, Distribution of log-cpm of all genes before and after normalization.

C, GSEA enrichment plots showing up-regulated gene sets in prim- or mono-AML specimens.

D, Heatmaps from GSEA analysis showing expression of each gene within a given gene set in ROSlow prim-AML (N=7) and ROSlow mono-AML (N=5). Red indicates relatively higher expression; Blue indicates relatively lower expression.

E, A heatmap showing expression pattern of apoptosis regulators in ROSlow prim-AML (N=7) and ROSlow mono-AML (N=5).

F, Overlaid dot and box plots showing mRNA expression of BCL2 in different FAB subtypes in the TCGA-AML dataset. Each dot represents a unique AML patient specimen. Box represents median +/- interquartile.

G, A heatmap showing expression pattern of apoptosis regulators in FAB-M0 (N=16), M1 (N=44), M2 (N=40) and M5 (N=21) subclasses of AMLs from the TCGA dataset.

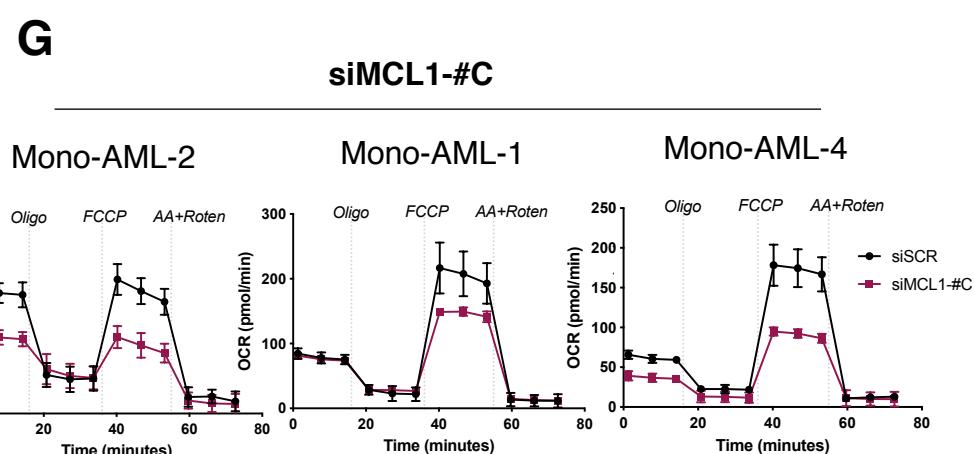
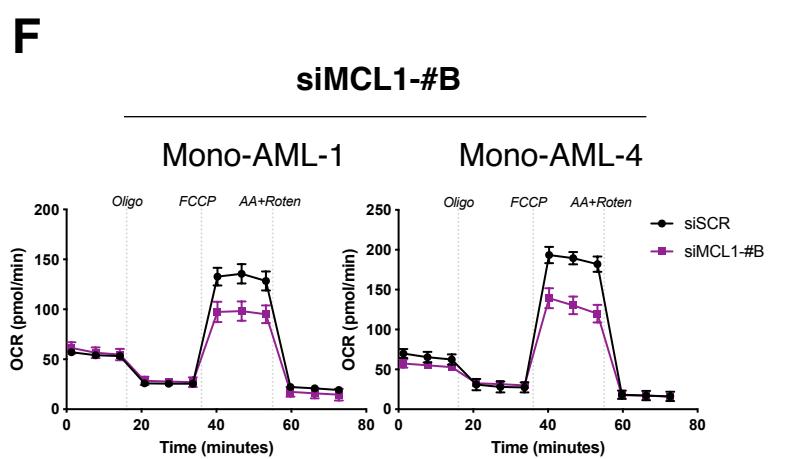
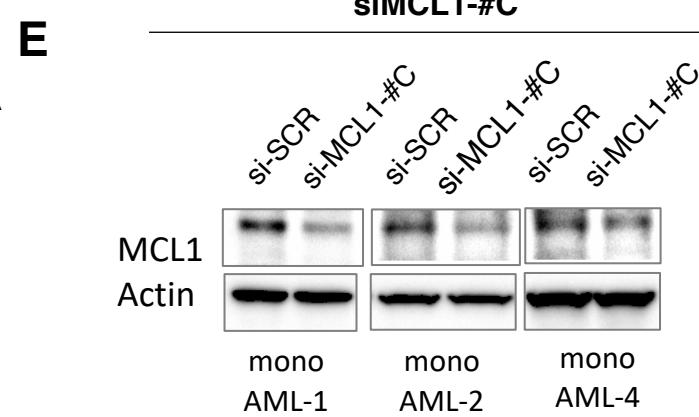
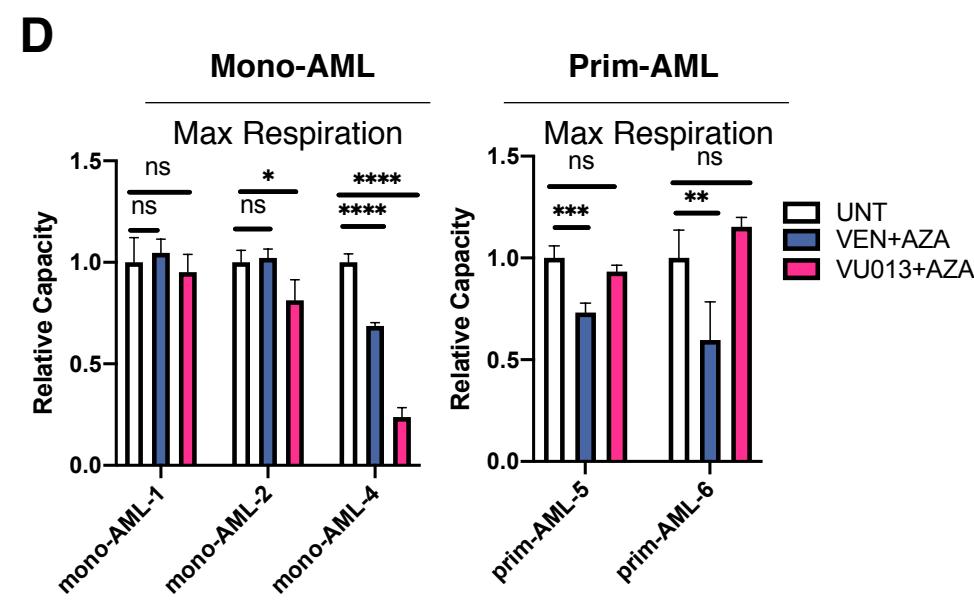
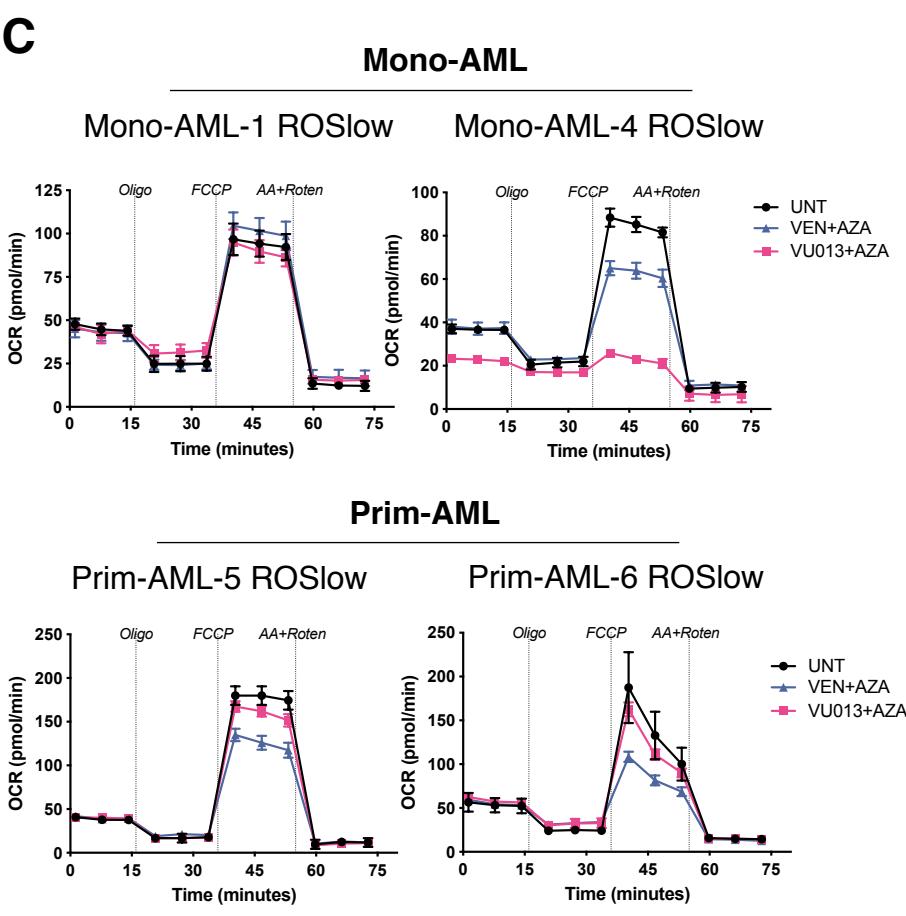
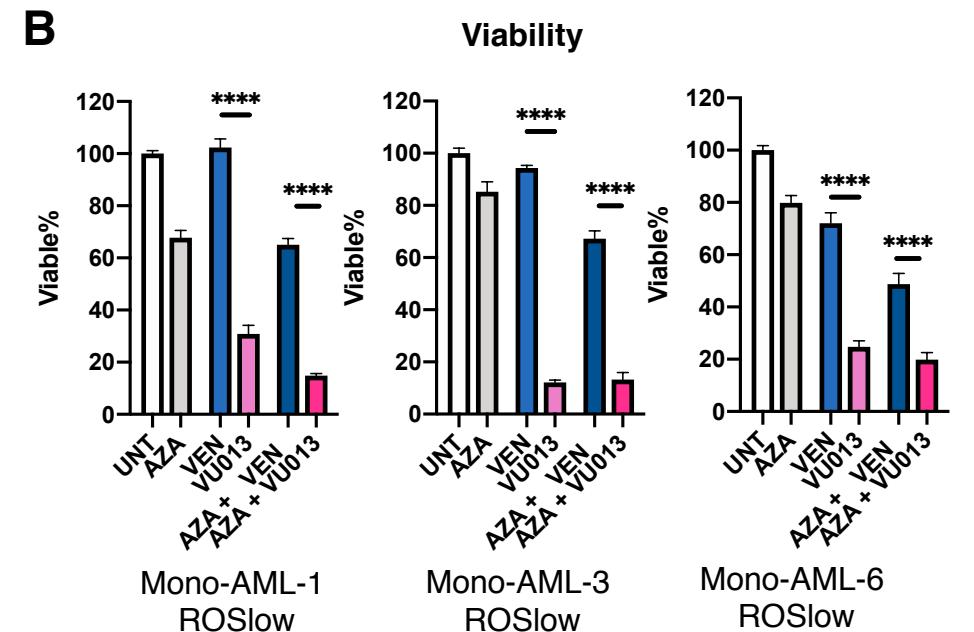
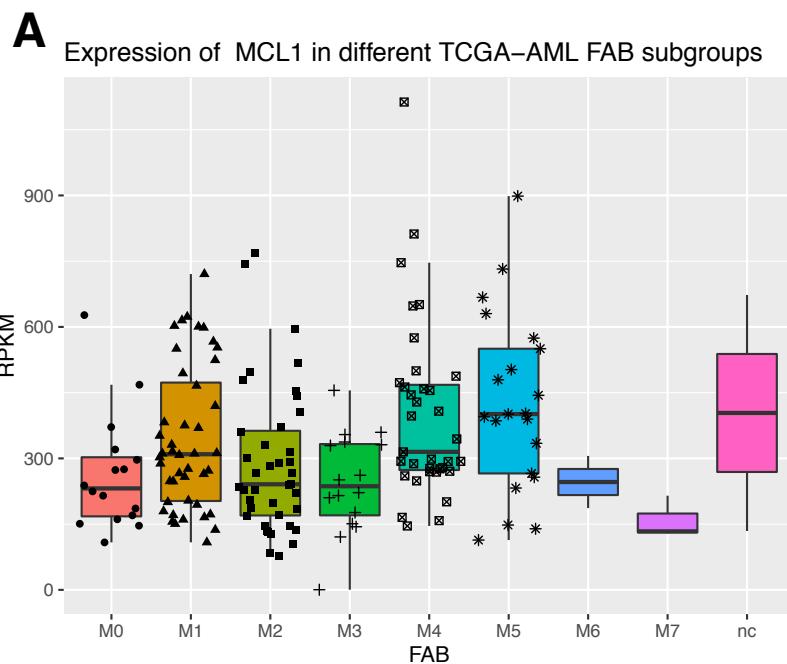
H, A heatmap showing expression pattern of apoptosis regulators along the monocytic developmental axis during human normal hematopoiesis. Raw data from GSE24759.

I, Trajectory of BCL2 expression change along the monocytic developmental axis during human normal hematopoiesis. Raw data from GSE24759. Each line represents a unique probe used to assess expression level of the targeted genes.

J, A heatmap showing expression pattern of apoptosis regulators along the monocytic developmental axis during murine normal hematopoiesis. Raw data from GSE60101.

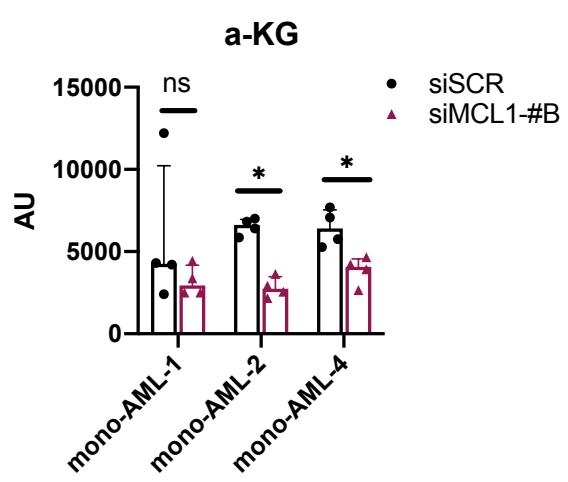
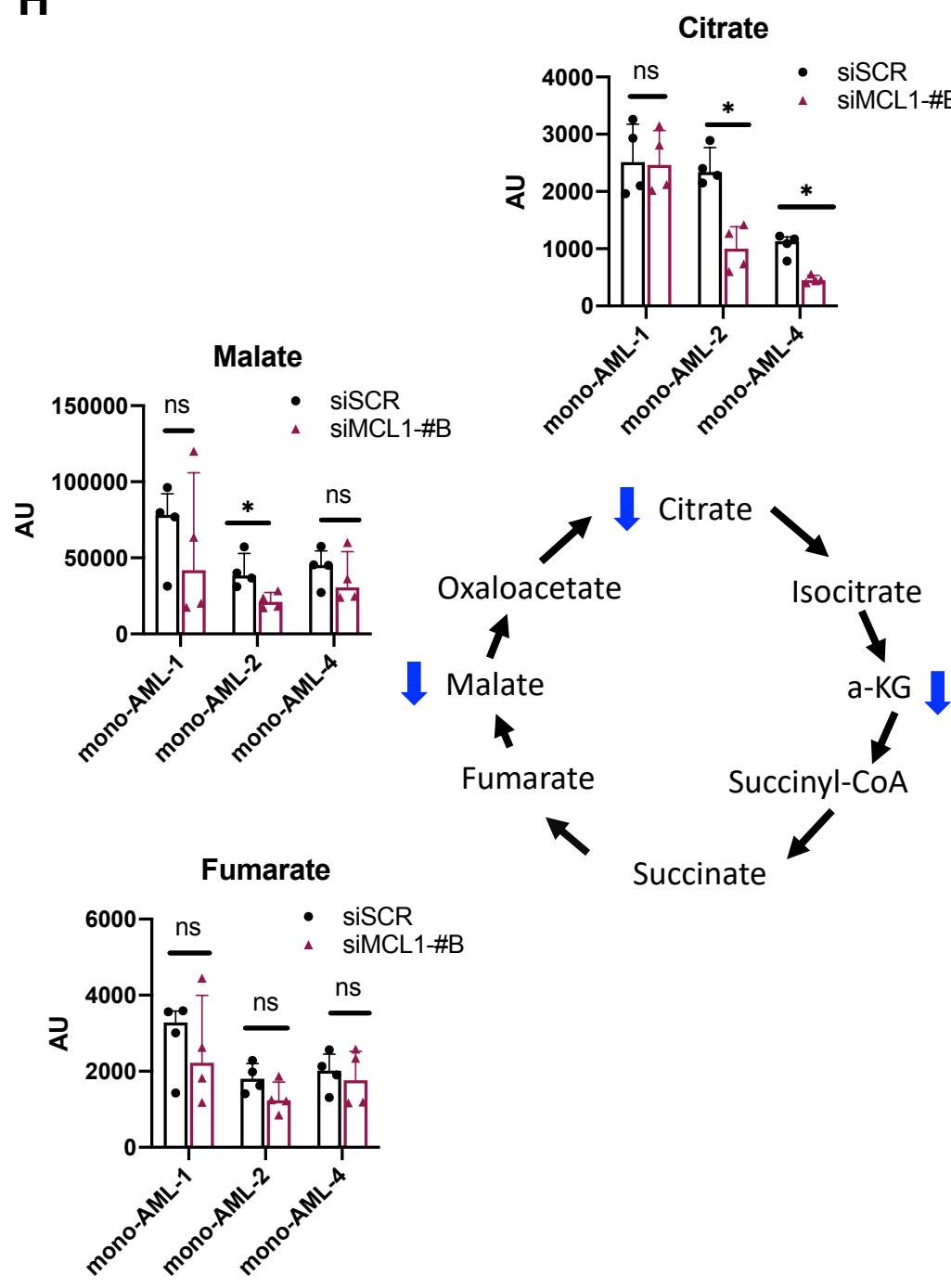
K, Trajectory of Bcl2 expression change along the monocytic developmental axis during murine normal hematopoiesis. Raw data from GSE60101.

Supplementary Figure S3

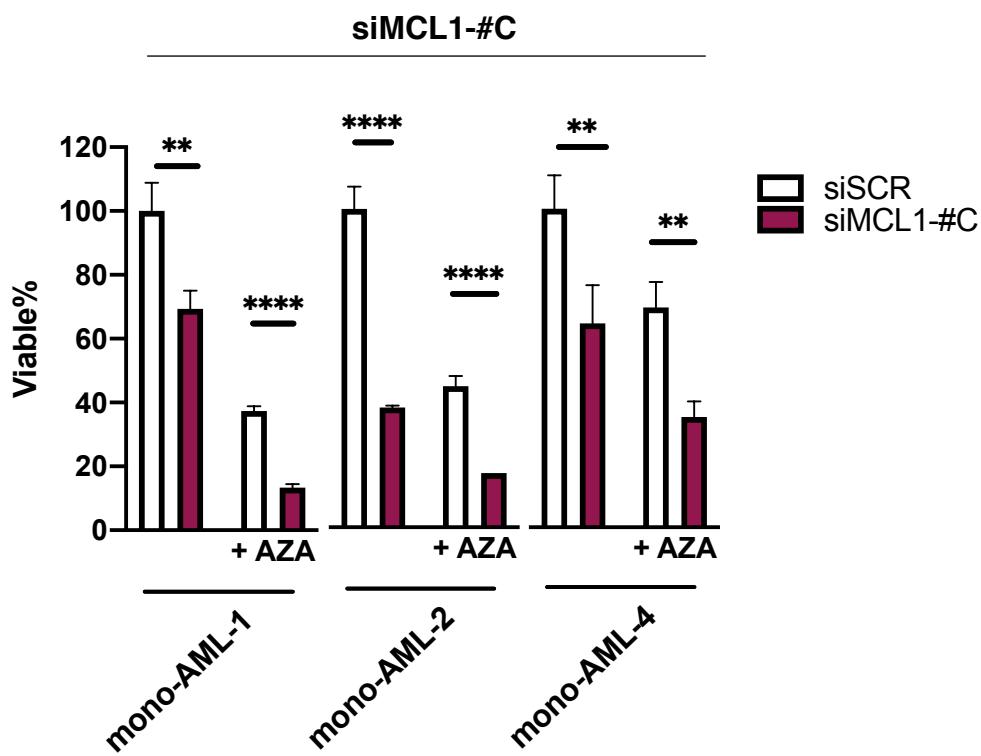


Supplementary Figure S3 continued

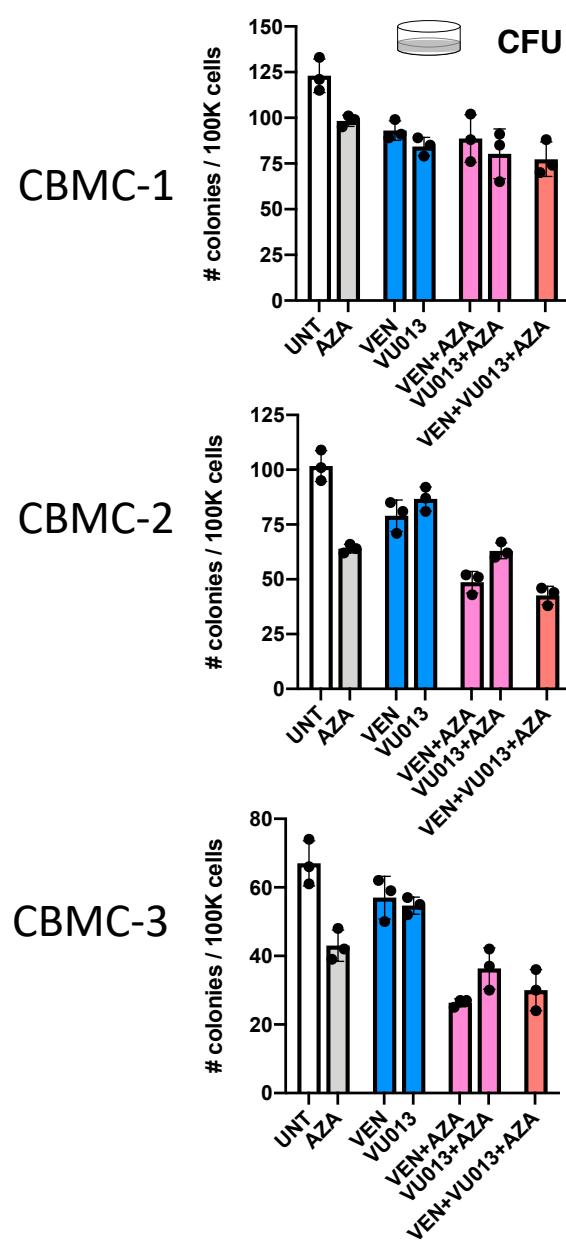
H



I



J



Supplementary Figure S3. Monocytic loses dependency on BCL2 but retains preferential reliance on MCL1.

A, Overlaid dot and box plots showing mRNA expression of MCL1 in different FAB subtypes in the TCGA-AML dataset. Each dot represents a unique AML patient specimen. Box represents median +/- interquartile

B, Relative viability of monocytic AML specimens treated 24 hours with 0.5uM VEN +/- 1.5uM AZA or 0.5 uM VU013 (VU661013) +/- 1.5uM AZA. Technical triplicates per group. Mean +/- SD. Two-tailed, unpaired t-test.

C, Oxygen consumption rate (OCR) curve from Seahorse Mito Stress Assay comparing impact of 0.5uM VEN + 1.5uM AZA and 0.5uM VU013 (VU661013) + 1.5uM AZA on OXPHOS activity of monocytic and primitive AML specimens. Technical replicates of five per data point. Mean +/- SD. Vertical dotted lines show injection times used in the Mito Stress Assay.

D, Relative impact of 0.5uM VEN + 1.5uM AZA and 0.5uM VU013 (VU661013) + 1.5uM AZA on maximal respiration rate of monocytic and primitive AML specimens calculated from the Seahorse Mito Stress Assay. Technical replicates of five per group. Mean + SD. Two-tailed, unpaired t-test.

E, Western blot results showing siMCL1-#C-mediated knock down of MCL1 at protein level.

F, OCR curve comparing OXPHOS activity in siMCL1-#B vs siSCR (siScramble) control monocytic AML. Technical replicates of five per data point. Mean +/- SD. Vertical dotted lines show injection times used in the Mito Stress Assay.

G, OCR curve comparing OXPHOS activity in siMCL1-#C vs siSCR (siScramble) control monocytic AML. Technical replicates of five per data point. Mean +/- SD. Vertical dotted lines show injection times used in the Mito Stress Assay.

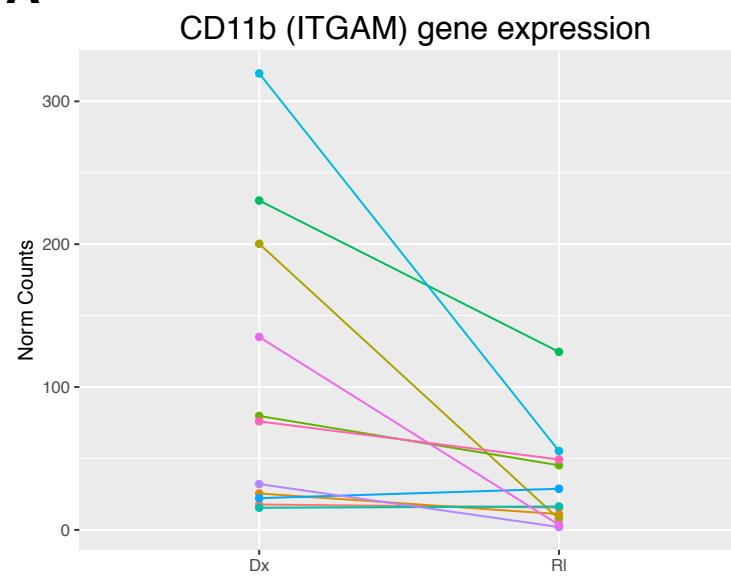
H, Quantification of TCA cycle intermediate metabolites in siMCL1-#B vs siSCR (siScramble) control monocytic AML. Technical replicates of four per data point. “AU” indicates Arbitrary units. Blue arrow indicates trending loss in siMCL1 relative to siSCR control. Median +/- Interquartile. Mann-Whitney test was used to determine significance.

I, Relative viability of monocytic AML cells with 48 hours exposure to siMCL1-#C or siSCR control, with or without presence of 1.5uM AZA. Technical triplicates per group. Mean +/- SD. Two-tailed, unpaired t-test.

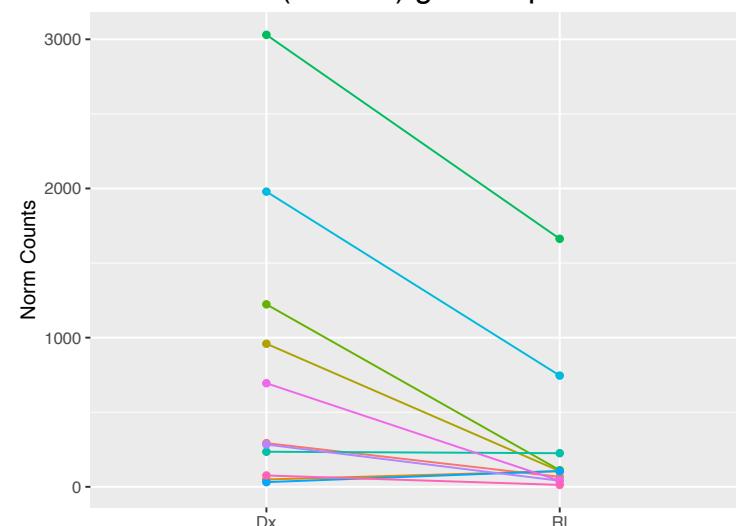
J, Colony-Forming Unit assay results showing stem/progenitor potential of normal cord blood mononuclear cells (CBMC) treated with various drug regimens. Technical triplicates.

Supplementary Figure S4

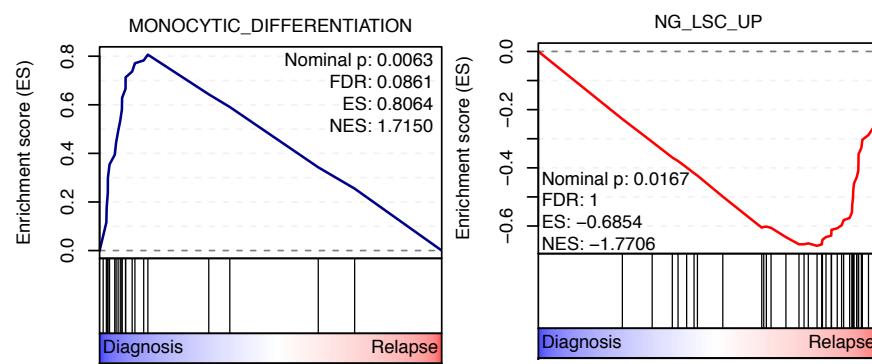
A



CD68 (LAMP4) gene expression



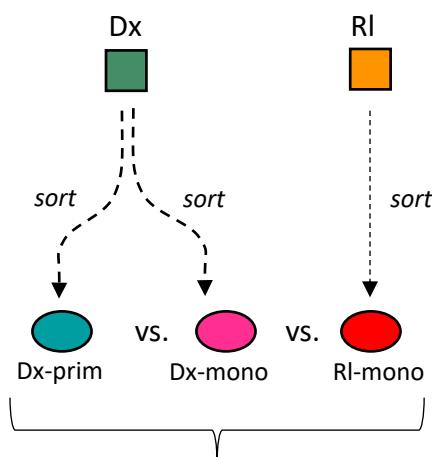
B



C

Patient	Stage	Phenotype	CD117	CD34	CD11b	CD14	CD64
			POS	NEG	POS	NEG	POS
Pt-1	Dx	primitive + mono	POS	NEG	POS	NEG	POS
	Relapse	primitive	POS	NEG	Dim	NEG	NEG
Pt-2	Dx	mono	NEG	NEG	POS	PP	POS
	Relapse	primitive	POS	POS	NEG	NEG	NEG
Pt-3	Dx	primitive	POS	POS	PP	NEG	PP
	Relapse	primitive	PP	PP	PP	NEG	NEG
Pt-4	Dx	primitive	NEG	NEG	Dim	NEG	Dim
	Relapse	primitive	NEG	NEG	POS	NEG	NEG
Pt-5	Dx	primitive	PP	NEG	PP	NEG	NEG
	Relapse	primitive	POS	NEG	POS	NEG	NEG
Pt-6	Dx	na	/	/	/	/	/
	Relapse	primitive	POS	POS	/	/	/

D



E

Pt-12

Mutation	Dx-prim	Dx-mono	RI-mono
NRAS.p.Q61K	0	35	51
SMC1A.p.R807H	42	0	0
IDH2.p.R140Q	70	48	46
SRSF2.p.P95R	50	44	47
TET2.p.L1721W	50	51	52

F

Pt-65

Mutation	Dx-prim	Dx-mono	RI-mono
KRAS.p.G13D	0	1	21
KRAS.p.G12V	0	<1*	31
EZH2.p.D185H	46	46	0

* Assumed pre-existing at <1% VAF

Supplementary Figure S4. Venetoclax + azacitidine selects out pre-existing monocytic subclones at relapse.

A, mRNA expression of monocytic marker gene CD11b and CD68 in paired diagnosis and chemo-relapsed AML patient specimens from the Shlush *et al.* study. Each pair of dots represents a unique AML patient specimen. Total of 11 pairs.

B, GSEA analysis of 11 paired diagnosis and relapse specimens from AML patients treated with conventional chemotherapy. The enrichment plots show down-regulation of monocytic differentiation gene set and up-regulation of LSC gene set at relapse in comparison to diagnosis. Raw data from Shlush *et al.*

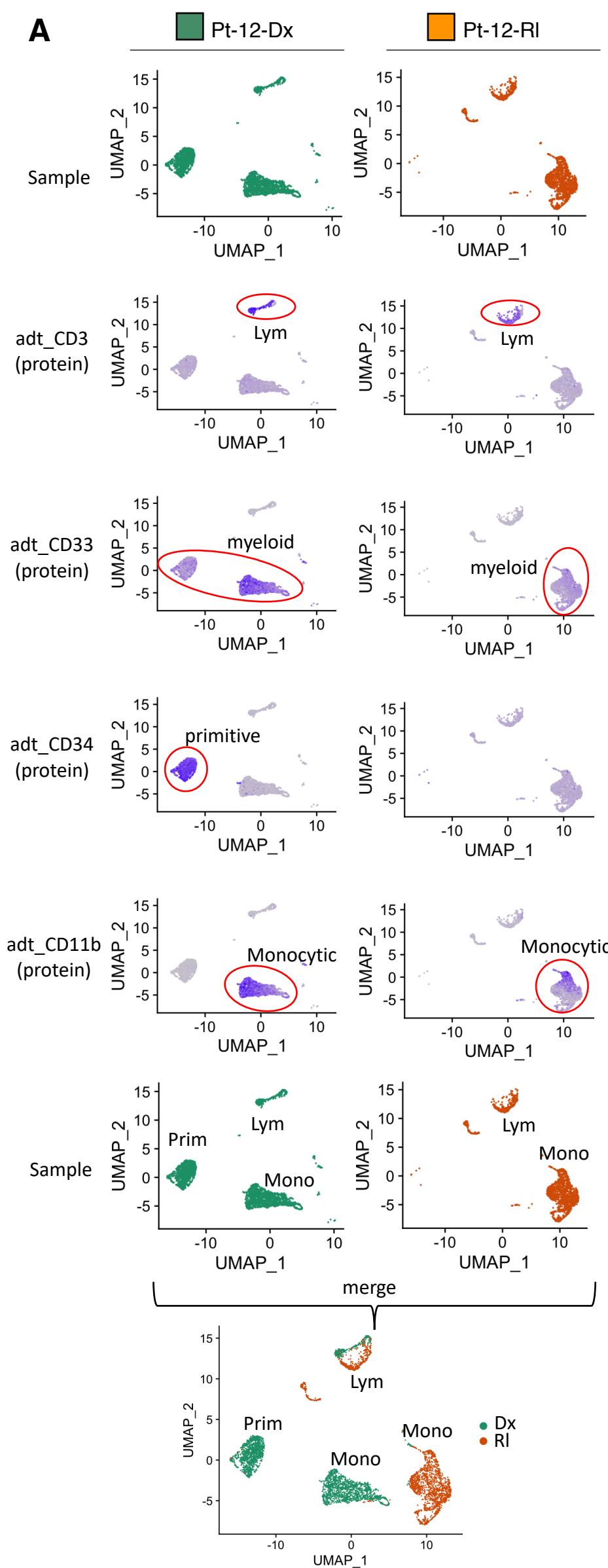
C, Clinical flow results of paired diagnosis (Dx) and relapse (Relapse) bone marrow specimens from 6 AML patients receiving conventional chemotherapy. Phenotype labels were interpreted from expression pattern of primitive markers CD117, CD34 and monocytic markers CD11b, CD14 and CD64. In general, negativity of CD117 and CD34 combined with positivity of CD11b, CD14 and CD64 were interpreted as monocytic; the opposite patterns were interpreted as primitive.

D, Sorting strategies for WES studies.

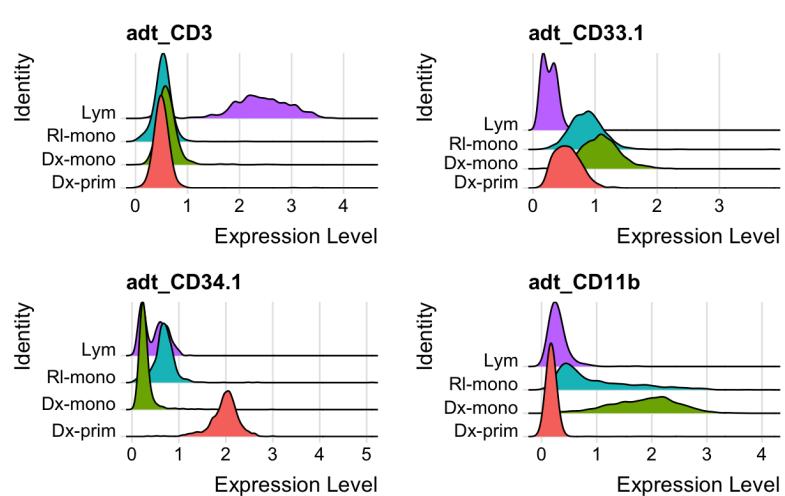
E, F, Mutation profile and variant allele frequency of each unique non-synonymous and cancer-related mutation in sorted subpopulations of cells including Dx-prim, Dx-mono and Relapse-mono. Colors highlight unique mutations that assigned clonal relationship among the subpopulations.

Supplementary Figure S5

A

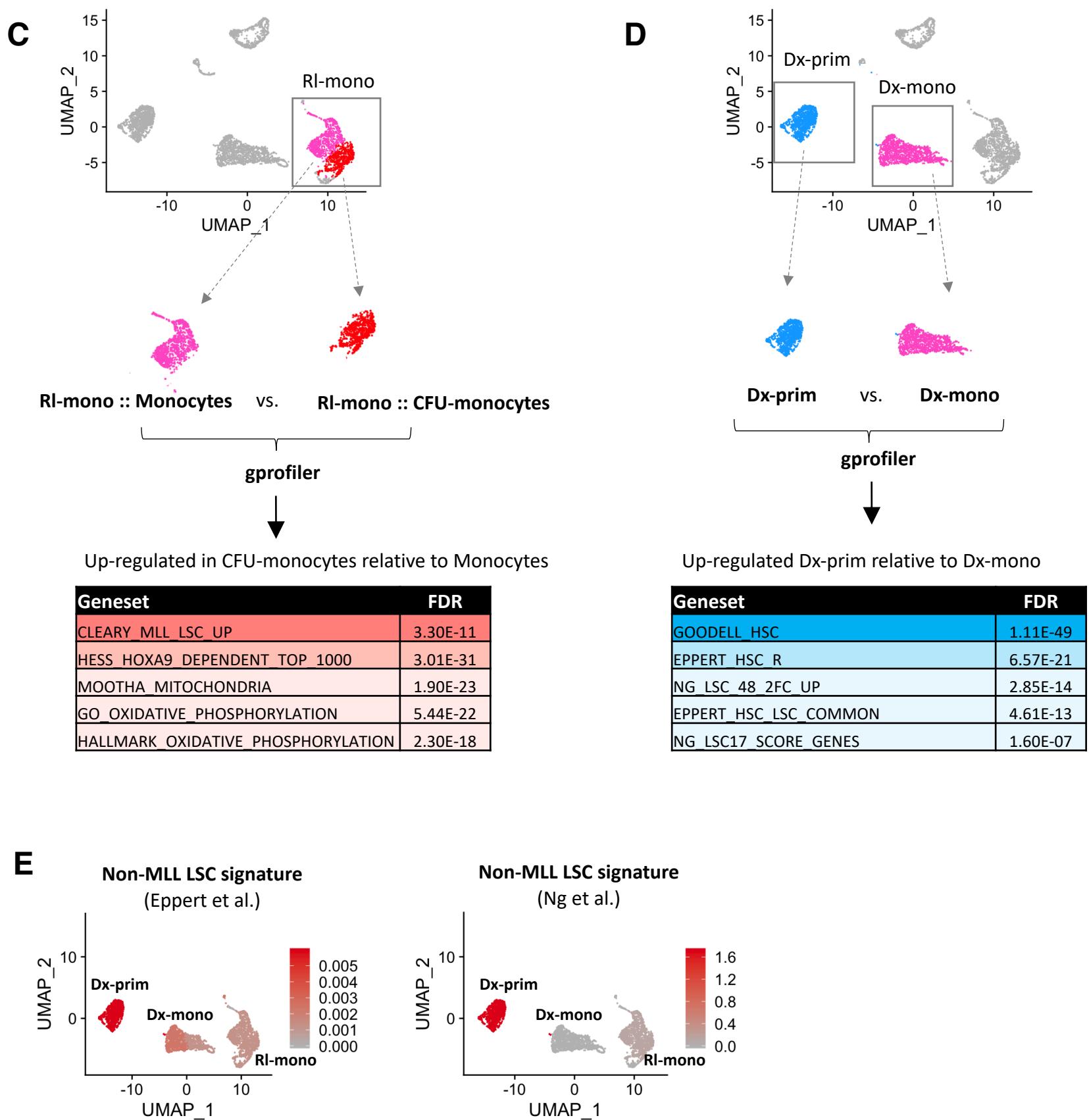


B



Supplementary Figure S5

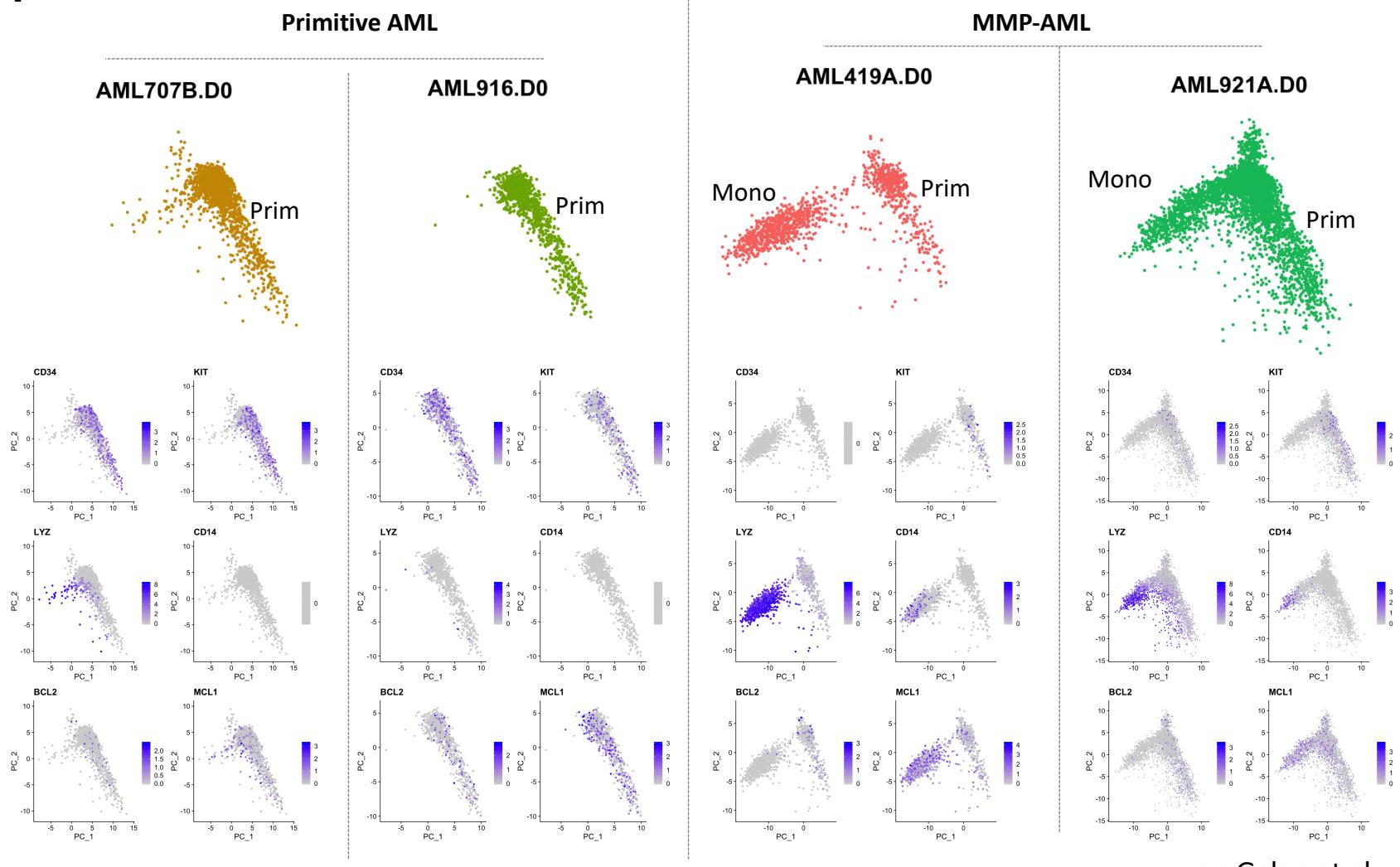
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Supplementary Figure S5

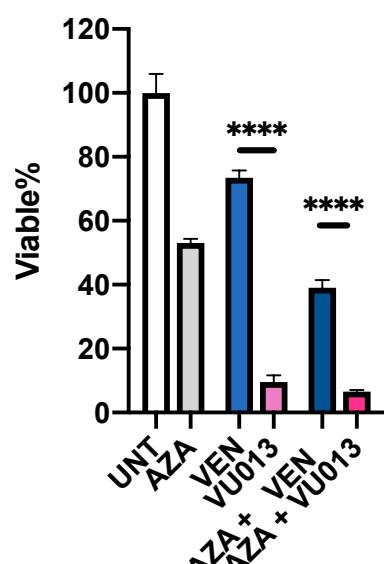
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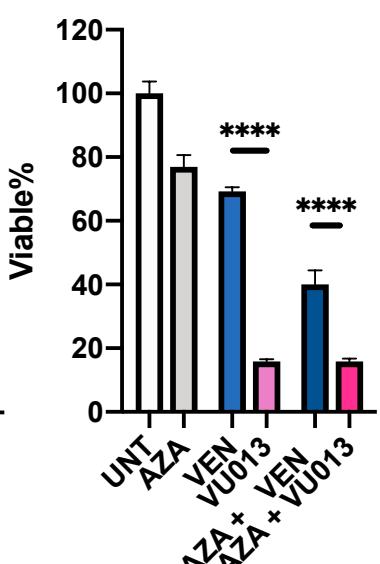


G

Viability

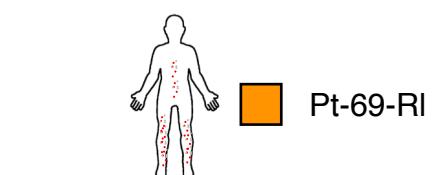


Pt-12-RI
ROSlow

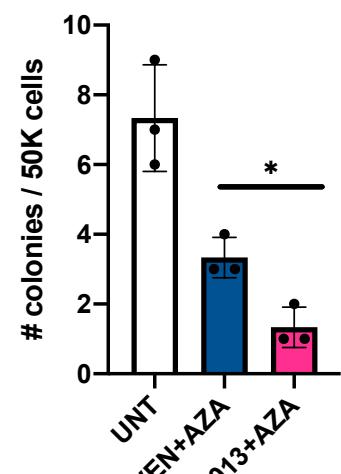
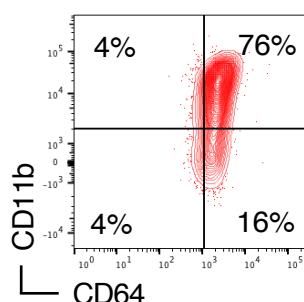
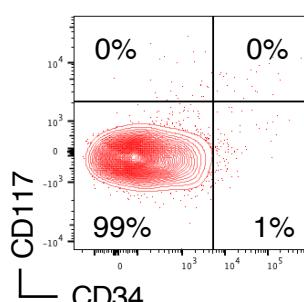
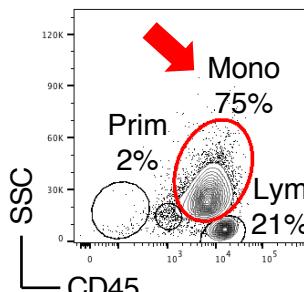


Pt-65-RI
ROSlow

H



CR → Relapsed BM



Pt-69-RI
ROSlow

Supplementary Figure S5. Monocytic disease at relapse has activated MLL-specific LSC programs and sustained reliance on MCL1.

A, UMAP plots of single cell data generated from CITE-seq analysis of paired diagnosis (Pt-12-Dx) and relapse (Pt-12-RI) specimens from Pt-12. Each cluster represents a subpopulation of biologically similar cells clustered by their transcriptome similarity. Each dot within each cluster represents a single cell. Protein expression of surface antigens CD3 (lymphoid), CD33 (myeloid), CD34 (primitive), and CD11b (monocytic) were measured using oligonucleotide-conjugated antibodies and their expression level in each single cell were overlaid on UMAP and used to initially assign identity of each cluster. The intensity of purple positively correlates to the expression of level of each antigen.

B, Ridge plots produced by Seurat analysis showing the expression pattern of CD3 (lymphoid), CD33 (myeloid), CD34 (primitive) and CD11b (monocytic) in different subpopulations of cells from Pt-12.

C, Gprofiler analysis results showing significantly upregulated gene sets in the “CFU-monocytes” subcluster relative to the “Monocytes” subcluster at relapse.

D, Gprofiler analysis results showing significantly upregulated gene sets in the “Dx-prim” cluster relative to the “Dx-mono” cluster at diagnosis.

E, Heatmaps showing relative expression of non-MLL LSC gene expression signatures at single cell resolution. Red indicates strong positive expression of the non-MLL LSC signatures; Gray indicates weak to none expression of the non-MLL LSC signatures.

F, Expression of primitive markers CD34, KIT/CD117, monocytic markers LYZ, CD14, and BCL2, MCL1 in the van Galen sc-RNAseq dataset. AML707B.D0 and AML916.D0 are classified as primitive AML due to their selective expression of primitive markers CD34, CD117 and lack of CD14, LYZ. AML419A.D0 and AML921A.D0 are classified as MMP-AML because they contain both primitive and monocytic subclusters as indicated by their expression pattern of primitive and monocytic markers. These classifications are consistent with van Galen et al. study. Notice the monocytic cluster within AML419A.D0 and AML921A.D0 have unique expression of MCL1 but not BCL2. D0 indicates day 0, *i.e.* diagnosis specimen.

G, Relative viability of monocytic AML specimens treated 24 hours with 0.5uM VEN + 1.5uM AZA or 0.5uM VU013 (VU661013) + 1.5uM AZA . The cells were obtained from relapsed specimen of Pt-12 and Pt-65. Technical triplicates per group. Mean +/- SD. Two-tailed, unpaired t-test.

H, Flow analysis of relapse specimen acquired from Pt-69. In the CD45/SSC plots, Mono, Prim and Lym gates identify monocytic, primitive and lymphocytic populations, respectively. The CD34/CD117 and CD64/CD11b plots show immunophenotype of the gated monocytic subpopulations in red. Arrows highlight populations of interest. Clinical information of the patient is listed in Supplementary Table S1.

I, Results of Colony Forming Unit (CFU) assay comparing impacts of 0.5uM VEN + 1.5uM AZA versus 0.5uM VU013 (VU661013) + 1.5uM AZA on the stem/progenitor function of mono-AML isolated from relapsed specimen of Pt-69. Mean + SD. Two-tailed, unpaired t-test.